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SERUM COMPOSITION OF FRESHWATER STINGRAYS (POTAMOTRYGONIDAE) ADAPTED TO FRESH AND DILUTE SEA WATER

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The osmotic adaptation of elasmobranch fishes to marine environments involves the retention of organic molecules, particularly urea, to maintain the osmotic pressure of body fluids at, or slightly in excess of, environmental levels. Recent discoveries that urea retention is lost in freshwater stingrays from South America (Thorson, Cowan and Watson, 1967; Junqueira, Hoxter and Zago, 1968) and is present in the coelacanth (Pickford and Grant, 1967; Lutz and Robertson, 1971), however, indicate that the mechanism is neither ubiquitous in nor restricted to elasmobranchs. The observations that serum urea levels are markedly reduced when marine or euryhaline elasmobranchs are subjected to dilutions in environmental salinity (Smith, 1931; Price and Creaser, 1967; Urist, 1962; Thorson, 1967) and that renal urea loss is increased (Smith, 1931; 1936) suggest, conversely, that freshwater stingrays might decrease urea loss when subjected to increased salinity and utilize this molecule in osmoregulation. Preliminary investigations of this possibility by Thorson (1970) failed to demonstrate elevated urea levels in the blood of fish adapted for short periods of time to increasing salinities. Clearly further investigations, employing more gradual adaptation, are needed.

From the point of view of inorganic electrolytes and osmolarity the freshwater stingrays have levels lower than marine elasmobranchs and comparable with those of teleosts (Thorson *et al.*, 1967; Junqueira *et al.*, 1968). Whether the mechanisms of ionic and osmotic regulation are also comparable is not known. While other elasmobranchs are known to enter fresh water (Smith, 1931) only the South American stingrays are permanent residents in this medium. In fact the group has probably lived in fresh water for millions of years. Larrazet (1886) described fossil stingrays from Tertiary deposits in the Rio Parana basin which Garman (1913) placed in his genus *Potamotrygon*. This long adaptation to fresh water of a predominantly marine group makes the river rays of considerable interest in terms of physiological evolution.

The purpose of the present investigation is twofold: to survey the major inorganic and organic serum constituents of freshwater stingrays in an attempt to

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elucidate the factors permitting such rays to live permanently in fresh water in contradistinction to all other known elasmobranchs, and to ascertain whether gradually-increased salinity affects these parameters. In addition, some data on electrolyte composition in other body fluids (pericardial and perivisceral) are presented.

Materials and Methods

The fish used in the present studies, reputedly from the Amazon basin, were obtained through a local tropical fish retailer (Connecticut Aquarium; East Haven, Connecticut) in late April, 1970. A total of 20 fish were acquired; twelve of these succumbed to infections prior to the initiation of experiments and data on eight fish are reported here. All were juveniles of small size, ranging in weight from 61 to 190 grams.

The stingrays were initially adapted to running, dechlorinated New Haven tap water at 25° C. Fish were maintained in separate 20 gallon aquaria and were fed a diet of live tubifex worms. The fish were kept under laboratory conditions for one and one half months before the initiation of experiments. At this time the surviving stingrays (13) were divided into two groups: 6 freshwater controls and 7 fish subjected to increased salinity. Salinity was raised by permitting Long Island Sound water (salinity 29‰) to slowly drip into the aquaria. Increases of 0.7‰ per day were accomplished in this manner. A preliminary test on a single fish indicated that osmoregulatory failure occurred at 20.6‰, and it was concluded that a salinity of 14.5‰, intermediate between this value and one isoosmotic with serum (based on the data of Thorson *et al.*, 1967), would be ideal for our purposes. Of the six remaining experimental fish, three succumbed to infections during the 20 days that salinity was being increased (at 7.0, 8.7 and 10.6‰); two of the control fish died also. At the time of autopsy there remained four freshwater controls and three experimentals at a salinity of 14.5‰. All fish save one of the experimentals appeared to be in excellent condition.

At autopsy the fish were anesthetized in MS 222 (one part to 1000 parts of water from the tank), sponged with distilled water, blotted dry, weighed, and the pericardical cavity was exposed. Pericardial fluid was collected in microhematocrit tubes, the pericardium was blotted dry, and the heart was punctured with fine scissors. Whole blood was drawn immediately for pH measurements and blood was taken for hematological studies. Blood was then collected in microhematocrit tubes, permitted to clot, and centrifuged. The abdomen was opened, perivisceral fluid was taken, and the livers removed, weighed, and frozen for shipment to Dr. Leon Goldstein, Brown University. Aliquots of serum were taken for total CO_2, Na+/K+ and chloride, and the remainder was frozen in tightly-corked vials at −20° C for subsequent analyses.

The methods and procedures used in the analysis of serum and other fluids were essentially identical to those developed for studies on *Fundulus heteroclitus* and described in detail in Pickford, Grant and Umminger (1969). Only microliter samples were required in most cases and all analyses were completed within one month of autopsy. Procedures for hematological studies were as described in Pickford, Srivastava, Slicher and Pang (1971).

Some difficulties of systematics

It was observed that our stingrays included a diverse assemblage of different forms in respect to external morphology. As it seemed possible that such morphological variability might be associated with physiological differences and as an aid in comparison of our data with other studies, an attempt was made to establish a sound nomenclatural designation for our fish. Our attempts to relegate specific names to the stingrays studied in the present report proved less than successful for several reasons: (1) uncertainty as to the applicability and status of the generic names within the family Potamotrygonidae; (2) presence within our sample of forms not readily assignable to any described species of river ray; (3) uncertainty as to the status of and relationships between any of the putative species of Potamotrygonidae; and (4) ignorance regarding the precise region(s) within the Amazon drainage from which our rays were collected. In view of the current interest in these fishes and, perhaps, as a spur to systematists to settle some of the taxonomic ambiguities in the river rays, some further discussion appears pertinent.

The first point to be made concerns the generic appellation *Potamotrygon* (Garman, 1877) frequently applied to the river rays. Of the five nominal genera in the family Potamotrygonidae three (*Elipesurus* (Schomburgk, 1843), *Paratrygon* (Dumereil, 1865) and *Disceus* (Garman, 1877)) have priority over *Potamotrygon*. Recently Castex (1968), after concluding that the type species of *Elipesurus*, *E. spinicaudata*, was identical to *Trygon brachyurus* Günther (a species generally placed in the genus *Potamotrygon*), proposed for reasons of simplicity that the genus *Elipesurus* be suppressed under the plenary powers of the International Commission on Zoological Nomenclature (I.C.Z.N.) in favor of *Potamotrygon*. Castex (1968) avoided use of the nominal genus *Paratrygon* used by Fowler (1948) on the grounds that identification of the type species (the "aiereba" of Marggrave) was not possible. In a reply to Castex, Bailey (1969) agreed with Castex's treatment of *Paratrygon* but suggested that *Elipesurus spinicaudata* was identical to *Disceus thayeri* Garman. Bailey thought it unwise to suppress *Elipesurus* and suggested that it should replace *Disceus*. Although both authors have stressed the fact that the type species of *Paratrygon* is unidentifiable we might note that Dumereil's description of the genus was based explicitly on a specimen (in the Munich museum) rather than Marggrave's figure of the "aiereba." Subject to rulings of the I.C.Z.N., identification of the Munich specimen could establish the identity of the type species of *Paratrygon* and make the genus available for use. While authors have consistently regarded *Disceus* and *Potamotrygon* (or their equivalents) as distinct from one another, one of the forms studied in the present report is intermediate in several respects between *Disceus* and *Potamotrygon* as defined by Garman (1913). In view of the nomenclatural uncertainty we might suggest, pending revisionary studies of the group, that future physiological studies on the river rays avoid indiscriminate use of generic names. Already Bailey (1969) has used the existence of the name *Potamotrygon* in the physiological literature (apparently based solely on Thorson *et al.* (1967) and Mathews (1966); Smith (1931) referred only to *Elipesurus* and Thorson *et al.* (1967) also referred to *Elipesurus* and *Disceus*) as an argument for its retention.

A comparison of our specimens with published descriptions of river rays (compiled in Garman, 1913) demonstrated that one form agrees with '*Potamotrygon*'

motoro and a second with *'P.' reticulatus*. A third form was the aforementioned intermediate between *'Potamotrygon'* and *'Disceus'* and three are apparently undescribed types of *'Potamotrygon.'* As little is known of geographic variation or species relationships in river rays and as we do not know the localities from which our fish were collected further systematic treatment is not possible. The physiological data presented here involves only three forms: three specimens of *'Potamotrygon' motoro* (one in fresh water and two in dilute sea water); four of an unidentified *'Potamotrygon'* (two specimens in fresh water, one in dilute sea water and one failing at 20.6‰); and one specimen of the intermediate in fresh water. Fortunately, we observed no consistent variability in the physiological parameters studied which could be correlated with morphological differences or sex and we feel

TABLE I

Hematology and collateral data on rays adapted to fresh or dilute sea water

	Fresh water	One half sea water	
		With sick fish	Sick fish omitted
Number	4	3	2
Hepatosomatic index	2.08 ± 0.16	1.56 ± 0.34	1.87
RBC (10^6/mm^3)	0.296 ± 0.032	0.253 ± 0.057	0.298
RBC length (μ)	17.65 ± 0.87	17.97 ± 0.69	17.30
RBC width (μ)	11.90 ± 0.60	11.60 ± 0.68	11.10
RBC nuclear length (μ)	7.66 ± 0.24	7.60 ± 0.21	7.40
RBC nuclear width (μ)	5.69 ± 0.57	5.10 ± 0.10	5.00
Thrombocytes (10^3/mm^3)	33.8 ± 1.3	35.0 ± 0.47	30.0
Leucocytes (10^3/mm^3)	5.12 ± 1.49	3.70 ± 1.26	4.95
Sm. lymphocytes (% WBC)	65.3 ± 6.4	70.0 ± 10.0	80.0
Lg. lymphocytes (% WBC)	8.5 ± 2.9	10.7 ± 4.7	13.5
Monocytes (% WBC)	13.8 ± 9.0	9.3 ± 7.9	1.5
Neutrophils (% WBC)	7.5 ± 4.8	5.0 ± 2.0	2.5
Eosinophils (% WBC)	5.0 ± 1.7	5.0 ± 2.9	2.5

justified in lumping forms for statistical comparison. The carcasses of all stingrays studied in the present investigation have been preserved and deposited in the fish collections of the Peabody Museum of Natural History, Yale University (Bingham Oceanographic Collection catalogue number 6995).

RESULTS

Hematology and collateral data

Data on the hematology and on collateral parameters of freshwater stingrays are presented in Table I. No significant effect of salinity was found on any hematological parameter studied nor was the hepatosomatic index or liver color affected by the salinity of the medium. There is an apparent correlation of hepatosomatic index and erythrocyte and leucocyte counts with health; the fish which had the lowest values for these parameters was markedly sluggish at autopsy and had very low levels of serum organic constituents (glucose, total carbohydrates, cholesterol, urea and proteins). As the possibility exists that the aberrant values of this fish

for collateral data and serum organic constituents might have been induced by high salinity rather than health, we have presented the data on these parameters both with the sick fish included and omitted (Tables I and III). Blood smears from most of our specimens revealed the presence of a bacterium similar to that responsible for hemorrhagic disease in *Fundulus heteroclitus* (*Aeromonas* sp.). There was no relationship between severity of infection and health; the sick fish had only a moderate infection while several heavily infected fish seemed to be in perfect health.

A comparison of our data on liver size with values on marine elasmobranchs reviewed by Olivereau and Leloup (1950) showed that our values were relatively low. Many factors including maturity, sex, diet and buoyancy functions affect the hepatosomatic index in elasmobranchs, however, and it is difficult to interpret our values in respect to those of marine species.

Red cell counts in river rays were in the range reported for marine elasmobranchs by Malassez (1872) and Saunders (1966); these levels are much lower than those of teleosts. Erythrocyte size averaged $17.8 \times 11.8\ \mu$; a value comparable with those reported for many marine elasmobranchs by Saunders (1966) but somewhat smaller than values reported for other batoids by Malassez (1872), Kisch (1951) and Saunders (1966). Our data on white cell counts and differential counts are in essential agreement with those of Saunders (1966) on marine elasmobranchs. We observed no basophils in our fish.

Survival in hyperosmotic media

Our preliminary data, based largely on the failure of a single healthy fish, indicated that juvenile stingrays are unable to survive at salinities in excess of 20.6‰. Earlier failures (at 7.0, 8.7 and 10.6‰) of experimental fish were associated with bacterial or fungal infections and control fish showed equivalent mortality. Three specimens reached a salinity of 14.5‰, and of these, two were still healthy.

Our data, such as they are, appear to conflict with those of Thorson (1970) who adapted several fish to salinities in excess of 20‰, one of which reached a final salinity of 32.3‰. Thorson did not report that any of his fish adapted to high salinities were at or near failure. Several factors could be responsible for the apparent discrepancy: size differences, differences in rate salinity increase and possible inter- or intraspecific differences. While Thorson's fish averaged 2.24 kilograms, ours were under 200 grams. It is possible that osmoregulatory failure proceeds more rapidly in small fish. Although we are not certain of the actual rate of salinity increase in Thorson's study (as he only gave the final salinity and days elapsed since the beginning of salinity increase), it is apparent that our fish were acclimated more gradually (*ca.* 0.7‰ per day *vs.* 5.1 to 21.6‰ per day). The possibility of large inter- or intraspecific differences in salinity tolerance exists. Both phenonena are evident in species of the teleost genus *Fundulus* that are restricted to fresh water (Griffith, 1972). Additional studies are necessary before the factors affecting salinity tolerance in river rays are fully understood.

Inorganic cations ($Na+$, $K+$, $Ca++$, $Mg++$)

Significant increases in serum sodium (21%), calcium (48%) and magnesium (51%) were observed in fish adapted to one half sea water when compared with

freshwater controls (Table II). Serum potassium was also somewhat higher in the experimental fish (21%) although the increase was not significant. It is of interest that serum sodium in the saline-adapted fish is iso-ionic with the environment (198.3 *vs.* 197.7 mEq/l; Tables II and V). Determinations of serum sodium on one failing fish at a salinity of 20.6‰ (Table IV) showed levels only slightly less than calculated environmental sodium concentrations (262 *vs.* 280 mEq/l). Although serum calcium and magnesium are elevated, both electrolytes are maintained at levels below the environment in dilute sea water.

Our data on serum cation levels in fresh water and in dilute sea water show minor differences from those reported previously (Thorson, 1970; Junqueira *et al.*,

TABLE II

Serum inorganic electrolytes and blood pH in rays (Potamotrygonidae) adapted to fresh or to one half sea water (salinity, 14.5‰)

	Fresh water	Half sea water	Per cent change
Number	4	3	
Na+ (mEq/l)	164.0 ± 5.6	198.3 ± 2.7	+20.9**
K+ (mEq/l)	4.45 ± 0.25	5.37 ± 0.38	+20.7
Ca++ (mEq/l)	3.04 ± 0.41	4.50 ± 0.12	+48.0*
Mg++ (mEq/l)	2.31 ± 0.17	3.49 ± 0.22	+51.1**
Cl− (mEq/l)	151.7 ± 5.0	183.1 ± 2.0	+20.7**
Total CO_2 (mM/l, equilibrated with 7% CO_2)	9.75 ± 0.79	11.35 ± 1.75	+16.4
HCO_3− (mEq/l, estimated)	7.95 ± 0.79	9.55 ± 1.75	+20.1
Total P (mM/l)	1.96 ± 0.67	2.59 ± 0.35	+32.1
Inorganic P (mM/l)	1.29 ± 0.24	1.74 ± 0.05	+34.9
Inorganic P (mEq/l at pH 7.3)	2.28 ± 0.42	3.07 ± 0.08	+34.6
pH (whole blood)	7.296 ± 0.025	7.323 ± 0.100	
Total cations mEq/l)	173.8 ± 5.1	211.7 ± 3.0	+21.8**
Total anions (mEq/l)	161.9 ± 5.3	195.7 ± 3.7	+20.9**
Cation excess (mEq/l)	11.9 ± 1.9	16.0 ± 1.9	+34.5
Total inorganic ions (mM/l)	332.1 ± 10.6	402.1 ± 6.5	+21.1**

* Significantly different from freshwater controls; $P < 0.05$.
** Significantly different from freshwater controls; $P < 0.01$.

1968). Serum sodium is somewhat higher in freshwater controls while potassium, calcium and magnesium are somewhat lower. These discrepancies might be due to differences between unstressed, laboratory-acclimated fish and wild rays subjected to recent capture. Thorson (1970) reported increases in serum sodium and magnesium at elevated salinities but these changes were not consistently related to the final salinity of the medium. Effects of salinity on calcium and potassium were equivocal in Thorson's study.

In general, our data support the statement of Thorson *et al.* (1967) that inorganic electrolyte levels are comparable to those of teleosts rather than marine elasmobranchs. Our data also suggest that the river rays are unable to actively excrete sodium. This would fit with the reported absence of the rectal gland in potamotrygonids (Goldstein and Forster, 1971), an organ implicated in active sodium excretion in marine elasmobranchs (Burger and Hess, 1960). Our rays possessed an organ in the anatomical position of the rectal gland, but histological

TABLE III

Serum organic constituents and osmolarity of stingrays adapted to fresh or one half sea water

	Fresh water	One half sea water	
		With sick fish	Sick fish omitted
Number	4	3	2
Organic P (mM/l)	0.59 ± 0.44	0.80 ± 0.35	1.07
Total cholesterol (mM/l)	2.80 ± 0.76	2.77 ± 0.87	3.64
Total free carbohydrates (as mM/l glucose)	4.91 ± 0.32	4.33 ± 1.12	4.72
Glucose (mM/l)	1.22 ± 0.17	0.68 ± 0.42	1.02
Non-glucose carbohydrates (as mM/l glucose)	3.72 ± 0.30	3.32 ± 0.55	4.21
Urea (mM/l)	1.08 ± 0.13	2.31 ± 0.77	2.95
Total protein (mg%)	829 ± 218	819 ± 290	1071
Fraction I (mg%)	64 ± 22	67 ± 29	85
Fraction II (mg%)	289 ± 123	239 ± 126	324
Fraction III (mg%)	207 ± 30	283 ± 78	361
Fraction IV (mg%)	118 ± 31	116 ± 47	157
Fraction V (mg%)	152 ± 40	110 ± 26	136
Percent albumin (V/total)	19.2 ± 3.7	15.1 ± 2.6	12.7
Calculated osmolarity†	338.1 ± 10.3	408.7 ± 6.0*	
Measured osmolarity (milliosmoles/liter	282.0 ± 16.8	477.7 ± 49.5*	
Calculated-measured osmolarity	+56.1	−69.0	

† Calculated osmolarity includes all inorganic ions, urea and total carbohydrates.
* Significantly different from freshwater controls; $P < 0.01$.

examination (Leon Goldstein, Roy Forster and William Doyle, personal communication) revealed that it was structurally unlike the rectal gland of marine elasmobranchs and was probably non-functional in salt secretion. While calcium and magnesium seem to be regulated somewhat, excretion of these divalent cations is urinary (Burger, 1967; Hickman and Trump, 1969). Freshwater stingrays are capable of active sodium uptake (Pang, Griffith and Kahn, 1972), although this uptake is inefficient at low environmental sodium levels. It appears that the pattern of sodium balance as well as serum levels of this electrolyte resembles that of freshwater teleosts (*cf.* Maetz, 1970).

Inorganic anions (Cl−, total CO_2, inorganic P)

We observed a significant increase in serum chloride (21%) and similar, but on account of high variance, non-significant increases in total CO_2 (16%) and inorganic phosphorus (35%) in fish adapted to dilute sea water (Table II). A further increase in serum chloride was observed in the fish failing at 20.6‰ (Table IV). The changes in chloride were similar to those in sodium in terms of percentage change and milliequivalents per liter. As chloride was maintained at levels lower than those of sodium, and as chloride in sea water is higher than sodium, serum chloride levels were not iso-ionic with the environment at elevated salinities.

A comparison of our data with that of Thorson (1970) showed similar levels of chloride in freshwater sera although inorganic phosphorus was lower in our

study. Thorson (1970) reported elevated chloride in saline media, in substantial agreement with our findings. Serum chloride levels in river rays are intermediate between those of freshwater teleosts and marine elasmobranchs (Holmes and Donaldson, 1969). Bicarbonate [estimated from total CO_2 and the known equilibration pressure of CO_2 using the nomograph of McLean (1938)] is higher than the levels found in marine elasmobranchs but is comparable to that found in *Fundulus heteroclitus* (Pickford *et al.*, 1969). Our levels of inorganic phosphorus are similar to those in marine elasmobranchs and are lower than typically found in teleosts (Holmes and Donaldson, 1969).

Serum urea

Although there was a strong trend towards an increase of serum urea (114%) in dilute sea water, low values for the sick fish prevented the difference from being significant (Table III). Were only healthy fish considered, small numbers would preclude demonstration of the significance of the increase (173%). The levels of urea in control fish (1.1 ± 0.1 mM/l) are in close agreement with the results of Thorson *et al.* (1967) (0.7 to 1.8 mM/l), and are but slightly lower than those of Junqueira *et al.* (1968) (1.9 ± 0.3 mM/l), thus reaffirming the unique hypouremia of the river rays among elasmobranchs. Marine and euryhaline elasmobranchs have urea concentrations ranging from 80 to 450 mM/l (Holmes and Donaldson, 1969). Our data correspond to the observation of Thorson (1970) that high salinities elicit small and irregular increases in serum urea.

While urea levels in river rays are very low, the fish possess the necessary ornithine-urea cycle enzymes for urea biosynthesis, albeit at activities much lower than in marine elasmobranchs (Goldstein and Forster, 1971). Assays of our fish livers for carbamyl phosphate synthetase, the rate limiting enzyme in the urea biosynthesis pathway in marine elasmobranchs, failed to demonstrate consistent effects of salinity on activity or correlations between serum urea and enzyme activity (Leon Goldstein, personal communication). The data clearly demonstrate that the river rays are unable to accumulate urea to levels comparable to those

TABLE IV

Distribution of sodium, potassium, and chloride in serum, pericardial fluid and perivisceral fluid of rays in fresh or dilute sea water (values in mEq/l)

Group	Ion	Serum	Pericardial fluid	Perivisceral fluid
Fresh water (N = 4)	Na+	164.0 ± 5.6	112.9 ± 14.4*	115.7 ± 14.4*
	K+	4.45 ± 0.25	5.48 ± 0.95	6.65 ± 1.27
	Cl−	151.7 ± 5.0	157.0 ± 6.0	159.7 ± 6.9
Half sea water (N = 3)	Na+	198.3 ± 2.7	147.8 ± 0.8**	156.3 ± 8.8**
	K+	5.37 ± 0.38	6.23 ± 2.14	6.90 ± 0.67
	Cl−	183.1 ± 2.0	199.7 ± 7.8	189.1 ± 2.1
Failing at 20.6‰ (N = 1)	Na+	261.8	218.0	223.4
	K+	12.6	13.5	11.5
	Cl−	222.4	267.1	286.4

* Significantly different from serum values; $P < 0.02$.
** Significantly different from serum values; $P < 0.001$.

in marine elasmobranchs, even when subjected to high salinities. Whether this inability is due solely to a failure to increase enzyme activities in response to salinity or reflects, as well, a failure of the kidney to actively resorb urea at high salinities is uncertain. Both factors appear to contribute to the low urea levels in fresh water (Goldstein and Forster, 1971).

Serum carbohydrates

We failed to detect significant changes in serum glucose, total carbohydrates, or non-glucose carbohydrates in stingrays adapted to dilute sea water (Table III). Our levels of glucose are lower than typical elasmobranch values (Kiermeir, 1939) although this could be related to activity patterns, feeding, handling, or differences in method (Kisch, 1929; Kiermeir, 1939). A correlation with health is apparent in the sick fish which had no detectable glucose. Our data show a striking discrepency between glucose (1.22 mM/1) and total carbohydrate concentrations (4.91 mM/1 as glucose) in control fish, suggesting that the predominant carbohydrates in stingray sera are not glucose. Similar relationships have been reported in the shark, *Scyliorhinus caniculus*, by Florkin (1936), Bocquet (1967) and Pérès and Rigal (1969). Parallel determinations of glucose and total reducing substances in the elasmobranchs *Raia erinacea* (Grant, 1964) and *Squalus acanthias* and the holocephalian *Hydrolagus collici* (Patent, 1970), however, failed to detect differences suggestive of significant levels of non-glucose carbohydrates. Although glucose appears to be the most abundant sugar in *Scyliorhinus caniculus* blood, Bocquet (1967) detected significant amounts of arabinose, fucose, xylose and rhamnose. Nixon (1965) found levels of meso-inositol in the related *S. stellaris* of 2.3 to 6.8 mg%. The identification of the non-glucose carbohydrates in the blood of river rays is a problem worthy of investigation.

Cholesterol and organic phosphorus

We observed no marked effect of salinity on measured serum cholesterol or total phosphorus, nor on derived values for organic phosphorus (Table III). Our cholesterol values are comparable to those reported in marine elasmobranchs by Mayer and Schaeffer (1913), Morris (1959), Sulya, Box and Gunter (1960), Urist and Van de Putte (1967) and Lauter, Brandenberger-Brown and Tram (1967). Our values for organic phosphorus were extremely variable (0.04 to 1.91 mM/1 in freshwater controls) and parameters which might be derived from these values (*e.g.* phospholipid and the ratio of phospholipid to cholesterol) were correspondingly variable and are omitted. A highly significant correlation ($r = 0.933$; $P < 0.005$) exists between organic phosphorus and one of the protein fractions. The relationship appears to be a linear function with origin at 0; suggesting that most of the organic phosphorus in stingray sera is protein bound.

Serum proteins

An interesting finding of our study is the presence of significant levels of serum albumin in freshwater stingrays. While some of the early literature (*e.g.* Nolf, 1907; Roche, Derrien and Fontaine, 1940; Cordier, Barnaud and Brandon, 1958) reported the presence of "albumin" in elasmobranchs, it is now apparent that marine elasmobranchs lack significant amounts of a serum component with the electrophoretic mobility of mammalian albumin (Deutsch and McShan, 1949;

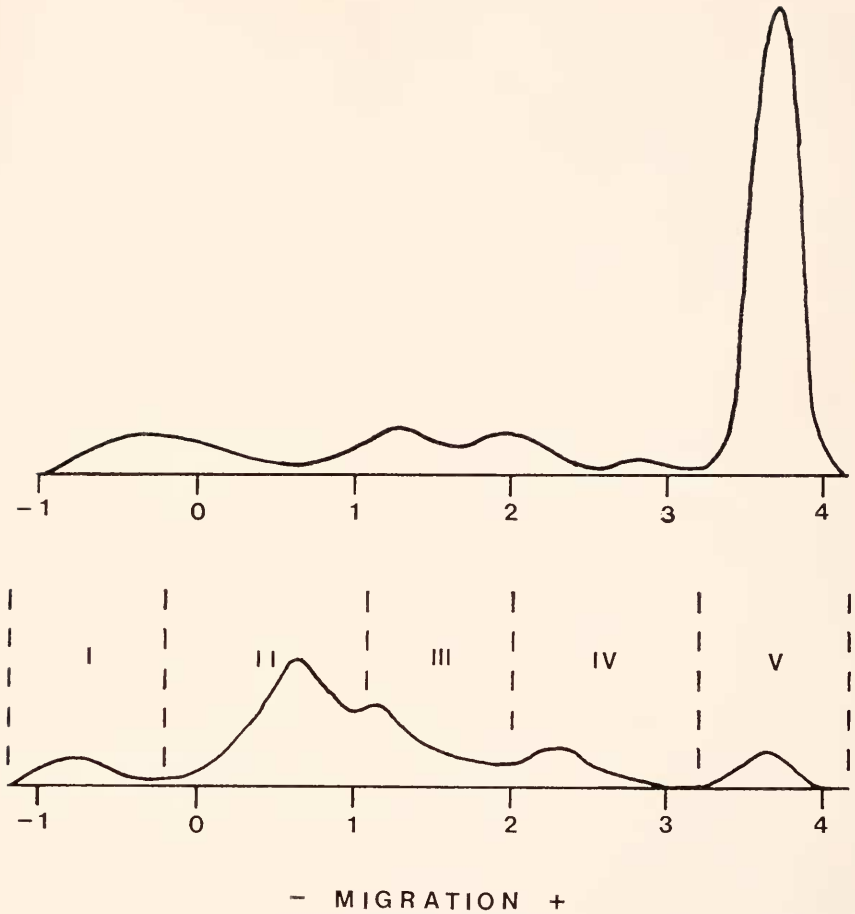

FIGURE 1. Comparison of densitometric tracings from protidograms of human (top) and freshwater stingray (bottom). Human pattern is from single application of standard Versatol (lot 0287050) and stingray pattern is from triple application of a freshwater control. Division of stingray pattern into five fractions is illustrated.

Irisawa and Irisawa, 1954; Sulya, Box and Gunter, 1961; Urist, 1961; Rasmussen and Rasmussen, 1967) or its physical properties (Urist, 1961). Urist and Van de Putte (1967) have used the absence of albumin as one of the distinguishing features of elasmobranchs, although a high mobility serum protein fraction is lacking in many teleosts as well (Pickford *et al.*, 1969). A statistical comparison of the positions of human albumin and the most mobile stingray fraction showed that the two were electrophoretically indistinguishable (Fig. 1). This comparison was between 18 stingray samples (including duplicates and several failing fish) and human standard sera (Versatol) run concomitantly, with the electropherograms positioned carefully on the densitometer so as to be directly comparable. Although the level of albumin was not high (58 to 231 mg%) it constituted a substantial fraction (10 to 26%) of the total protein.

Total protein levels (314 to 1430 mg%; Table III) are below those reported previously in river rays by Thorson *et al.* (1967) (1100 to 2300 mg%) but are similar to those found by Junqueira *et al.* (1968) (700 to 1300 mg%). Junqueira *et al.* (1968), using electrophoretic techniques, found five major protein zones. Our results are similar (Fig. 1). We found no differences in the position or distribution of the protein fractions which could be related to morphological type. A close correlation of one of the fractions (fraction II, Fig. 1) with organic phosphorus suggests that this component is a phosphorus-containing protein. We failed to detect any effect of salinity on total protein, on any individual fraction, or on the ratio of albumin to total protein. Nevertheless, it is worth speculating that the presence of albumin is one of the factor permitting the river rays to inhabit dilute media.

Serum osmolarity and total osmoticallly-active substances

Significant increases in both measured serum osmolarity (69%) and osmolarity calculated from the sum of the measured osmotically-active substances (21%) were observed in fish adapted to dilute sea water in comparison to those in fresh water (Table III). A rather large discrepancy between measured and calculated osmolarities was observed; the latter being 56.1 milliosmoles greater than the former in freshwater fish. Karhausen (1962) has pointed out that such discrepancies can occur as a consequence of ion-complexing substances in the blood or factors affecting salt dissociation such as temperature, pH or ionic concentrations.

In the present study it was found that the difference between measured and calculated osmolarities was reversed in dilute sea water; measured osmolarity being 69.0 milliosmoles greater than calculated values compared to 56.1 milliosmoles less in fresh water. This may be attributed to large increases in unmeasured serum components in the fish at the high salinity. The possibility that trimethylamine oxide, which like urea is retained by marine elasmobranchs at high levels, accounts for much of the unexplained increase in osmolarity would seem to be discounted by Thorson (1970) who found negligible amounts of this compound in saline-adapted river rays.

Thorson *et al.* (1967) give values for serum osmolarity (301 to 320 milliosmoles) which agree with our freshwater data in terms of levels (247 to 317), but differ in that they agree closely with calculated osmolarities. Junqueira *et al.* (1968) give somewhat higher osmolarities (350 ± 32 milliosmoles), but an insufficient number of parameters were determined to estimate total osmotically active substances. We believe that our observed difference between measured and calculated osmolarities in freshwater control fish is due to a physico-chemical property of stingray sera. Using identical techniques on *Fundulus heteroclitus* this laboratory has consistently found that measured osmolarity slightly exceeds calculated values based on a comparable number of serum parameters (Pickford *et al.*, 1969; Srivastava and Pickford, 1972; Umminger, 1969).

Blood pH and cation excess

Environmental salinity was without effect on either blood pH (which ranged from 7.145 to 7.481) or on the difference between the sums of the measured cations and anions (which ranged from 7.5 to 19.0 mEq/l). Neither parameter

was correlated with total CO_2, inorganic phosphorus, total serum proteins or albumin; serum constituents potentially involved in acid-base balance. We found a barely significant negative correlation ($r = 0.832$; $P < 0.05$) between blood pH and serum cation excess. The possibility that the two are correlated in this way through differences in blood lactic acid concentrations may be inferred from the studies of Piiper and Baumgarten (1969) who suggested that increases in cations would be necessary to maintain electrical neutrality in the blood of acidotic *Scyliorhinus stellaris* with low blood pH and high blood lactic acid. Our values for blood pH are similar to those reported for marine elasmobranchs by Heinemann and Hodler (1953), Green and Hoffman (1953) and Murdaugh and Robin (1967). Our values for cation excess are somewhat lower than those observed in the teleost *Fundulus heteroclitus* by Pickford *et al.* (1969).

TABLE V

Electrolyte concentrations in the Amazon River and in experimental tanks

Parameter	Amazon River†		Fresh water	Half sea water††
	Mean	Range		
Salinity (ppm)	44.75	37–59	100	14500
Sodium (mM/l)	0.047	0.030–0.068	0.150	197.7
Potassium (mM/l)	0.034	0.026–0.048	0.016	4.20
Calcium (mM/l)	0.196	0.136–0.312	0.167	4.30
Magnesium (mM/l)	0.048	0.021–0.073	—	22.5
Chloride (mM/l)	0.072	0.062–0.088	0.241	230.5

† Data after Clarke (1924); based on four analyses at different parts of river basin.
†† Values for other parameters estimated from salinity.

Perivisceral and pericardial fluids

Data are presented in Table IV for sodium, potassium and chloride of serum, perivisceral fluid and pericardial fluid for stingrays in fresh water, dilute sea water and failing at a salinity of 20.6‰. It is apparent that sodium and chloride of the three fluids are elevated in dilute sea water. This rise is not evident in potassium although levels of this ion were high in the failing fish. Of interest is the fact that both pericardial and perivisceral fluids are markedly hyponatremic, yet iso- or slightly hyperchloremic to serum in all media. These data are in agreement with Thorson *et al.* (1967), who also noted that the other cations measured ($K+$, $Mg++$, $Ca++$) in these fluids were at lower levels than in the serum. These data suggest that both pericardial and perivisceral fluids have a large cation deficit in contrast to the serum where there is a cation excess; a finding similar to observations on marine elasobranchs by Bernard, Wynn and Wynn (1966), Rodnan, Robin and Andrus (1962) and Murdaugh and Robin (1967).

DISCUSSION

In adapting to fresh water, the river rays of the family Potamotrygonidae have developed several physiological characteristics which set them apart from marine elasmobranchs. Most apparent is the elimination of urea retention as an osmoregu-

latory mechanism. Marine elasmobranchs have serum urea levels ranging from 209 to 453 mM/l (Holmes and Donaldson, 1969). Euryhaline species sampled in fresh water have concentrations ranging from 81 to 180 mM/l (Smith, 1931; Urist, 1962; Thorson, 1967). In striking contrast, the river rays have urea levels of 1 to 2 mM/l; concentrations which are, at most, tripled by adaptation to moderately high salinities. It would appear that urea retention in elasmobranchs is a specific adaptation to marine environments, subject to modification in response to dilute media through physiological mechanisms to only a limited extent, but labile in an evolutionary sense. The probably independent acquisition in the coelacanth (Pickford and Grant, 1967; Lutz and Robertson, 1971) and the certainly independent acquisition of the urea retention mechanism in the frog *Rana cancrivora* (Gordon, Schmidt-Nielsen and Kelly, 1961) suggest that urea retention is an "obvious" way for aquatic vertebrates to cope with the osmoregulatory problems inherent in maintaining a moderately low serum specific ion content in a medium with much higher electrolyte concentrations.

The question might be asked as to why the teleost fishes did not adopt the urea retention habitus while while in sea water, particularly in light of recent demonstrations that all of the enzymes of the ornithine-urea cycle are present in teleosts (Huggins, Skutch and Baldwin, 1969; Read, 1971). The answer may lie in an advantage inherent in the teleost mechanism of osmoregulation in sea water (*i.e.*, drinking sea water and excreting salts extrarenally; *cf.* Smith, 1930) in adapting to increases and decreases in environmental salinity. An as yet untested corollary of this hypothesis is the possibility, assuming that urea retention is metabolically inexpensive relative to drinking sea water and excreting ions, and assuming that urea tolerance is not a sufficient deterrent, that marine teleosts which have not been subjected to changes in environmental salinity for geologically long periods of time (*e.g.* bathybenthic and bathypelagic groups) may retain urea for osmoregulatory purposes. Of particular interest would be species which are ovoviviparous, viviparous, or have encapsulated eggs, developments which might be interpreted as consequences of urea retention (Smith, 1936; 1953; Price and Daiber, 1967).

A second feature making the river rays distinctive is the regulation of serum inorganic electrolytes at reduced levels compared to marine elasmobranchs. An analogous difference in normal levels of serum electrolytes may be found between marine and freshwater teleosts although the difference is not as marked in this group. Euryhaline teleosts maintain their serum electrolytes within narrow limits irrespective of the adaptation medium (Pickford *et al.*, 1969). In both elasmobranchs and teleosts it seems likely that the final serum electrolyte levels are the result of a balance between the expense of maintaining ion gradients which, unchecked, would result in salt loss in fresh water and salt gain in sea water, and the ability of tissues to tolerate changes in their ionic milieu. In both taxonomic groups the balance points differ between freshwater and marine species.

A third unique characteristic of the Potamotrygonidae among elasmobranchs is the presence of serum albumin. It is possible that the binding of ions by this protein is a mechanism of decreasing the osmotic gradient between the fish and its environment while maintaining the specific ion content of the serum within reasonable values. A function of serum albumin in adaptation to fresh water has

also been suggested for the teleosts (Roche, Derrien and Chouaiech, 1939; Drilhon and Fine, 1957), although it is clear that many marine teleosts have substantial amounts of an albumin-like protein (Sulya *et al.*, 1961; Morris, 1959) and that albumin plays no role in the adaptation of *Fundulus* species to fresh water (Pickford *et al.*, 1969; Griffith, 1972).

Finally, the adaptation of river rays to fresh water has proceeded to such an extent that they are no longer able to tolerate sea water. Failure to withstand high salinities is apparently due to an inability to excrete monovalent salts (sodium and chloride) which accumulate to high levels in fish living in dilute sea water. The loss of a functional rectal gland which would be of no use in a salt deficient environment possibly accounts for the inability of the river rays to excrete monovalent salts. Their failure to accumulate and/or retain urea in saline media is probably of secondary importance in preventing adaptation to high salinities.

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Summary

1. Juvenile freshwater stingrays of the family Potamotrygonidae are unable to survive in salinities in excess of 20.6‰ when gradually acclimated.

2. No differences were observed in blood pH or hematological parameters when fish adapted to a salinity of 14.5‰ were compared with freshwater controls.

3. Significant increases were found in serum sodium (21%), chloride (21%), calcium (48%), and magnesium (51%). Increases in total CO_2 (16%), potassium (21%), and inorganic phosphorus (35%) were not significant on account of high variance. Serum osmolarity increased 69%.

4. There was no apparent effect of salinity on serum total cholesterol, organically bound phosphorus, or total carbohydrates. Glucose contributed only 25% of the latter.

5. Serum urea was low (1.1 mM/l) as previously reported, and the trend to increase in a saline environment was not osmotically significant.

6. Freshwater stingrays are unique among elasmobranchs in possessing significant amounts of a protein with the electrophoretic mobility of human serum albumin. There was no significant change in this fraction or in serum total protein in fish adapted to a saline medium.

LITERATURE CITED

Bailey, R. M., 1969. Comment on the proposed suppression of *Elipesusus spinicaudata* Schomburgk (Pisces) ZN(S) 1825. *Bull. Zool. Nomencl.*, **25**: 133.

Bernard, G. R., R. A. Wynn and G. G. Wynn, 1966. Chemical anatomy of the pericardial and perivisceral fluids of the stingray, *Dasyatis americana*. *Biol. Bull.*, **130**: 18–27.

Bocquet, J., 1967. Identification de quelques oses du plasma d'un sélacien *Scylliorhinus canicula*. *C. R. Soc. Biol., Paris*, **161**: 836–840.

Burger, J. W., 1967. Problems in the electrolyte economy of the spiny dogfish, *Squalus acanthias*. Pages 177–185 in P. W. Gilbert, R. F. Mathewson and D. P. Rall, Eds., *Sharks, Skates and Rays*. The Johns Hopkins Press, Baltimore.

Burger, J. W., and W. Hess, 1960. Function of the rectal gland in the spiny dogfish. *Science*, **131**: 670–671.

Castex, M. N., 1968. *Elipesurus* Schomburkg 1843 (Pisces): proposed suppression under the plenary powers ZN(S) 1825. *Bull. Zool. Nomencl.*, **24**: 353–355.

Clark, F. W., 1924. The data of geochemistry. *U.S. Geol. Sur. Bull.*, **770**: 1–841.

Cordier, D., R. Barnaud and A. M. Brandon, 1958. Etude sur le proteinemie de la roussette (*Scyllium canicula* L.). Influence du jeune. *C. R. Soc. Biol., Paris*, **151**: 1912–1915.

Deutsch, H. F., and W. H. McShan, 1949. Biochemical studies on the blood plasma proteins XII. Electrophoretic studies of blood serum proteins of some lower animals. *J. Biol. Chem.*, **180**: 219–234.

Drilhon, A., and J. M. Fine, 1957. Les albumines chez quelques especes de poissons. *C. R. Acad. Sci., Paris*, **245**: 1676–1679.

Dumereil, A., 1865. Elasmobranches. In *Histoire Naturelle des Poissons*, Volume I, Part 2. Libraire Encyclopedique de Roret, Paris, 720 pp.

Florkin, M., 1936. Sur la glycemie plasmatique vraie d'un selacien (*Scyllium canicula*). *Bull. Classe Sci. Acad. Roy. Belg.*, **22**: 1185–1188.

Flower, H. W., 1948. Os peixes de água doce de Brasil. *Arquivos de Zool. do Estado de Sao Paulo*, **6**: 1–204.

Garman, S., 1877. On the pelvis and external sexual organs of selacians with especial reference to the new genera *Potamotrygon* and *Discecus*. *Proc. Boston Soc. Natur. Hist.*, **19**: 197–214.

Garman, S., 1913. The plagiostoma (sharks, skates and rays). *Mem. Mus. Comp. Zool., Harvard*, **36**: 1–515.

Goldstein, L., and R. P. Forster, 1971. Urea biosynthesis and excretion in freshwater and marine elasmobranchs. *Comp. Biochem. Physiol.*, **39B**: 415–421.

Gordon, M. S., K. Schmidt-Nielsen and H. M. Kelley, 1961. Osmotic regulation in the crab-eating frog, (*Rana cancrivora*). *J. Exp. Biol.*, **38**: 659–678.

Grant, W. C., 1964. Effects of insulin and glucagon on blood glucose levels in the skate, *Raia crinacea*. *Amer. Zool.*, **4**: 325–326.

Green, J. W., and J. F. Hoffman, 1953. A study of isotonic solutions for the erythrocytes of some teleosts and elasmobranchs. *Biol. Bull.* **105**: 289–295.

Griffith, R. W., 1972. Studies on the physiology and evolution of killfishes of the genus *Fundulus*. *Ph.D. dissertation, Yale University*, 470 pp.

Heineman, H. O., and J. E. Holder, 1953. Composition of body fluids in elasmobranchs. *Bull. Mt. Desert Is. Biol. Lab.*, **4**: 46–47.

Hickman, C. P., and B. F. Trump, 1969. The kidney. Pages 91–239 in W.S. Hoar and D. J. Randall, Eds., *Fish Physiology*, Volume I. Academic Press, New York.

Holmes, W. N., and E. M. Donaldson, 1969. The body compartments and the distribution of electrolytes. Pages 1–89 in W. S. Hoar and D. J. Randall, Eds., *Fish Physiology*, Volume I. Academic Press, New York.

Huggins, A. K., G. Skutch and E. Baldwin, 1969. Ornithine-urea cycle enzymes in teleostean fish. *Comp. Biochem. Physiol.*, **28**: 587–602.

Irisawa, H., and A. F. Irisawa, 1954. Blood serum proteins of the marine Elasmobranchii. *Science*, **120**: 849–851.

Junqueira, L. C. U., G. Hoxter and D. Zago, 1968. Observations on the biochemistry of fresh water rays and dolphin blood serum. *Rev. Brasil Pesqui. Med. Biol.*, **1**: 225–226.

Karhausen, L. R., 1962. Osmolarity. *Nature*, **194**: 1234–1235.

Kiermeir, A., 1939. Über den blutzucher der süsswasserfische. *Z. Vergl. Physiol.*, **27**: 460–491.

Kisch, B., 1929. Blutzuckeruntersuchen bei selaciern. *Biochem. Z.*, **211**: 270–291.

Kisch, B., 1951. Erythrocytes in fishes. *Exp. Med. Surg.*, **9**: 125–137.

Larrazet, A., 1886. Des pièces de la peau de quelques sélaciens fossiles. *Bull. Soc. Geol., France*, **1886**, III Ser. **14**: 255–277.

Lauter, C. J., E. A. Brandenberger-Brown and E. G. Tram, 1967. Composition of the

plasma lipoproteins of the spiny dogfish, *Squalus acanthias*. *Comp. Biochem. Physiol.*, **24**: 243–247.

Lutz, P. L., and J. D. Robertson. 1971. Osmotic constituents of the coelacanth *Latimeria chalumnae* Smith. *Biol. Bull.*, **141**: 553–560.

Maetz, J., 1970. Mechanisms of salt and water transfer across membranes in teleosts in relation to the aquatic environment. Pages 3–29 in G. K. Benson and J. G. Phillips, Eds., *Hormones and the Environment, Memoirs Society Endocrinology*, Volume XVIII.

Malassez, L., 1872. De la numération des globules rouges du sang chez les mammifères, les oiseaux, et les poissons. *C. R. Acad. Sci., Paris*, **75**: 1528–1531

Mathews, M. B., 1966. The molecular evolution of cartilage. *Clin. Orthop.*, **48**: 267–283.

Mayer, A., and G. Schaeffer, 1913. Recherches sur la teneur des tissus en lipoides (quatrième mémoire). Teneus en lipoides des globules et du sérum sanguin. *J. Physiol. Pathol. Gen.*, **15**: 984–998.

McLean, F. C., 1938. Application of the law of chemical equilibrium (law of mass action) to biological problems. *Physiol. Rev.*, **18**: 495–523.

Morris, B., 1959. The proteins and lipids of the plasma of some species of Australian fresh and salt water fish. *J. Cell. Comp. Physiol.*, **54**: 221–230.

Murdaugh, H. V., and E. D. Robin, 1967. Acid-base metabolism in the dogfish shark. Pages 249–264 in P. W. Gilbert, R. F. Mathewson and D. P. Rall, Eds., *Sharks, Skates and Rays*. The Johns Hopkins Press, Balitmore.

Nixon, D. A., 1965. The glycogen and meso-inositol concentrations in the muscle and liver of the elasmobranch *Scyliorhinus stellaris*. *Pubbl. Staz. Zool. Napoli*, **34**: 515–520.

Nolf, P., 1907. Quelques observations concernant le sang des animaux marins. *Arch. Inter. Physiol.*, **4**: 98–116.

Olivereau, M., and J. Leloup, 1950. Variations du rapport hepato-somatique chez la roussette (*Scyllium canicula* L.) au cours du developpement et de la reproduction. *Vie Milieu*, **1**: 377–420.

Pang, P. K. T., R. W. Griffith and N. Kahn, 1972. Electrolyte regulation in the freshwater stingrays (Potamotrygonidae). *Federation Proc.*, **31**: 344.

Patent, G. J., 1970. Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch (*Squalus acanthias*) and a holocephalian (*Hydrolagus collici*). *Gen. Comp. Endocrinol.*, **14**: 215–242.

Pérès, G., and A. Rigal, 1969. De l'effet exerce par l'ablation du cervelet sur la glycemie de la petite roussette (*Scylliorhinus canicula*). *C. R. Acad. Sci., Paris*, **D268**: 2926–2928.

Pickford, G. E., and F. B. Grant, 1967. Serum osmolality in the coelacanth, *Latimeria chalumnae*, urea retention and ion regulation. *Science*, **155**: 568–570.

Pickford, G. E., F. B. Grant and B. L. Umminger, 1969. Studies on the blood serum of the euryhaline cyprinodont fish, *Fundulus heteroclitus*, adapted to fresh or to salt water. *Trans. Conn. Acad. Arts Sci.*, **43**: 25–70.

Pickford, G. E., A. K. Srivastava, A. M. Slicher and P. K. T. Pang, 1971. The stress response in the abundance of circulating leucocytes in the killfish, *Fundulus heteroclitus* I. The cold shock sequence and the effects of hypophysectomy. *J. Exp. Zool.*, **177**: 89–96.

Piiper, J., and D. Baumgarten, 1969. Blood lactate and acid-base balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. *Pubbl. Staz. Zool. Napoli*, **37**: 84–94.

Price, K. S., and E. P. Creaser, 1967. Fluctuations in two osmoregulatory components, urea and sodium chloride, of the clearnose skate *Raja eglanteria* Bosc 1802. I. Upon laboratory modification of external salinities. *Comp. Biochem. Physiol.*, **23**: 65–76.

Price, K. S., and F. C. Daiber, 1967. Osmotic environments during development of dogfish, *Mustelus canis* (Mitchell) and *Squalus acanthias*, and some comparison with skates and rays. *Physiol. Zool.*, **40**: 248–260.

Rasmussen, L. E., and R. A. Rasmussen, 1967. Comparative protein and enzyme profiles of the cerebrospinal fluid, extradural fluid, nervous tissue, and sera of elasmobranchs. Pages 361–379 in P. W. Gilbert, R. F. Mathewson and D. P. Rall, Eds., *Sharks, Skates and Rays*. The Johns Hopkins Press, Baltimore.

Read, L. J., 1971. The presence of high ornithine-urea cycle enzyme activity in the teleost, *Opsanus tau*. *Comp. Biochem. Physiol.*, **39**: 409–413.

Roche, J. Y. Derrien and M. S. Choucaiech, 1939. Le precipitation de proteines seriques par la sulfate d'ammonium. Le serum des vertebres marins (reptiles et poissons de divers ordres). *C. R. Soc. Biol., Paris,* **130**: 1301–1306.

Roche, J., Y. Derrien and M. Fontaine, 1940. Pression osmotique et poids moleculaire des albumines et des globulines serique des sélaciens et des cyclostomes et classification zoologique. *Bull. Soc. Chim. Biol.,* **22**: 395–406.

Rodnan, G. P., E. D. Robin and M. H. Andrus, 1962. Dogfish coelomic fluid: I. Chemical anatomy. *Bull. Mt. Desert Is. Biol. Lab.,* **4**: 69–70.

Saunders, D. C., 1966. Elasmobranch blood cells. *Copeia,* **1966**: 348–351.

Schomburgk, R. H., 1843. Fishes of British Guiana. Part 2. Pages 129–214 in *Jardine's Naturalist's Library,* Volume 40, Edinburgh, Scotland.

Smith, H. W., 1930. The absorption and excretion of water and salts by marine teleosts. *Amer. J. Physiol.* **93**: 480–505.

Smith, H. W., 1931. The absorption and excretion of water and salts by the elasmobranch fishes I. Fresh water elasmobranchs. *Amer. J. Physiol.,* **98**: 279–295.

Smith, H. W., 1936. The retention and physiological role of urea in the Elasmobranchii. *Biol. Rev.,* **11**: 49–92.

Smith, H. W., 1953. *From Fish to Philosopher,* Little Brown and Co., Boston, 264 pp.

Srivastava, A. K., and G. E. Pickford, 1972. The effects of hypophysectomy on the blood serum of male killifish, *Fundulus heteroclitus,* in salt water. *Gen. Comp. Endocrinol.,* in press.

Sulya, L. L., B. E. Box and G. Gunter, 1960. Distribution of some blood constituents in fishes from the Gulf of Mexico. *Amer. J. Physiol.,* **199**: 1177–1180.

Sulya, L. L., B. E. Box and G. Gunter, 1961. Plasma proteins in the blood of fishes from the Gulf of Mexico. *Amer. J. Physiol.,* **200**: 152–154.

Thorson, T. B., 1967. Osmoregulation in fresh-water elasmobranchs. Pages 265–270 in P. W. Gilbert, R. F. Mathewson and D. P. Rall, Eds., *Sharks, Skates and Rays.* The Johns Hopkins Press, Balitmore.

Thorson, T. B., 1970. Freshwater stingrays, *Potamotrygon* spp.: Failure to concentrate urea when exposed to saline medium. *Life Sciences,* **9** (part II): 893–900.

Thorson, T. B., C. M. Cowan and D. E. Watson, 1967. *Potamotrygon* spp.: Elasmobranchs with low urea content. *Science,* **158**: 375–377.

Umminger, B. L., 1969. Physiological studies on supercooled killifish (*Fundulus heteroclitus*) II. Serum organic constituents and the problem of supercooling. *J. Exp. Zool.,* **172**: 409–424.

Urist, M. R., 1961. Calicum and phosphorus in the blood and skeleton of the Elasmobranchii. *Endocrinology,* **69**: 778–801.

Urist, M. R., 1962. Calcium and other ions in blood and skeleton of Nicaraguan freshwater shark. *Science,* **137**: 984–986.

Urist, M. R., and K. A. Van de Putte, 1967. Comparative biochemistry of the blood of fishes. Pages 271–285 in P. W. Gilbert, R. F. Mathewson and D. P. Rall, Eds., *Sharks, Skates and Rays.* The Johns Hopkins Press, Baltimore.